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(54) Title: SUSTAINED RELEASE COMPOSITIONS FOR DELIVERY OF GROWTH FACTORS

(57) Abstract

Biodegradable films comprising a polylactic acid/polyglycolic acid copolymer, a therapeutically effective amount of a polypeptide growth factor, and a carrier are provided. The films may be affixed to the outer surface of an implantable or prosthetic device such as a screw, pin, plate, rod or artificial joint component. The films and rods are useful therapeutically, such as within methods of enhancing repair of bone fractures.

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Description

SUSTAINED RELEASE COMPOSITIONS FOR DELIVERY OF GROWTH FACTORS

Technical Field

The present invention relates generally to growth factors, and more specifically to biodegradable films loaded with growth factors and the therapeutic use of these films.

Background of the Invention

The growth and development of cells of higher organisms are dependent upon a variety of polypeptide growth factors. These growth factors vary in their specificity for target cells, creating a complex interplay that controls normal development and maintenance of tissue homeostasis. Growth factors have also been implicated in the development of proliferative disorders such as cancer and atherosclerosis.

Polypeptide growth factors can be grouped into families on the basis of structural homology and/or specificity. These families include the epidermal growth factor (EGF) family, which includes, in addition to EGF itself, transforming growth factor alpha (TGFα) and vaccinia growth factor (VGF). The fibroblast growth factor (FGF) family includes at least five members (reviewed by Thomas, Trends Biochem. Sci. 13: 327-328, 1988), including acidic and basic FGFs (aFGF and bFGF), int-2, hst/KS3 and FGF5. The platelet derived growth factor (PDGF) family includes homo- and hetero-dimers of the component A and B chains, the A chain occuring in several alternatively spliced forms (Tong et al., Nature 328: 619-621, 1987). The insulin-like growth factors, IGF I and IGF II (also known as somatomedin C and somatomedin A, respectively), are single chain polypeptides with a high degree of homology to insulin. Transforming growth

factor beta (TGFβ) is a dimer of 12.5 kDa polypeptide chains. The colony stimulating factors (CSFs) are characterized by their ability to influence the growth and development of hematopoietic precursor cells. Growth factors are reviewed by Tauber and Tauber, Nucl. Med. Biol. 14: 407-419, 1987.

While knowledge of growth factor activity has been obtained primarily through *in vitro* experiments, there is a growing body of data on the actions of growth factors *in vivo*. For example, PDGF has therapeutic applications for the treatment of injuries which require the proliferation of fibroblasts or smooth muscle cells to heal. More specifically, *in vivo*, PDGF normally circulates stored in the alpha granules of platelets. Injury to arterial endothelial linings causes platelets to adhere to the exposed connective tissue and release their granules. In this regard, PDGF has been shown to be active in promoting wound healing in several animal models and in clinical studies. For instance, Lynch et al. (Proc. Natl. Acad. Sci. USA 84: 7696-7700, 1987) disclose the use of a combination of insulin-like growth factor I and purified PDGF to promote wound healing. The two growth factors showed a synergistic effect in promoting the healing of dermal wounds in pigs. Lynch et al. (J. Clin. Peridontol. 16: 545-548, 1989) also found that a combination of PDGF and IGF I promotes bone and cementum formation in a dog model of periodontitis. In addition, Greenhalgh et al. (Am. J. Pathol. 136: 1235-1246, 1990) demonstrated enhanced healing of full-thickness skin wounds in genetically diabetic mice treated with recombinant PDGF as compared to control animals. PDGF also appears to participate in the initiation of fracture repair by stimulating mesenchymal cell proliferation and the synthesis of intramembranous bone (Joyce et al., 36th Annual Meeting, Orthopaedic Research Society, February 5-8, 1990, New Orleans, LA). Robson et al. (Lancet 339: 23-25, 1992) disclose the use of PDGF BB for the treatment of

chronic pressure ulcers. Antoniades et al. (U.S. Patent No. 5,035,887) disclose the use of combinations of interleukin 1 and PDGF or IGF I to promote healing of external wounds.

However, there remains a need in the art for delivery systems suited for the long-term, topical administration of growth factors such as PDGF. This need is due in part to the instability of certain polypeptide growth factors. For example, PDGF is sensitive to proteolysis (Hart et al., Biochemistry 29: 166-172, 1990; U.S. Patent Application Serial No. 07/557,219) and denaturation. The plasma half-life of PDGF has been found to be as short as two minutes in an animal model (Bowen-Pope et al., Blood 64: 458-469, 1984), and ^{125}I-TGFβ was found to disappear from the plasma with an initial $t_{1/2}$ of 2.2 minutes in rats (Coffey et al., J. Clin. Invest. 80: 750-757, 1987). In general, PDGF must be applied to a wound site on a daily basis, thereby limiting its use in fracture healing or other internal applications.

Thus, there is a need in the art for therapeutic compositions which are suitable for sustained delivery of growth factors. There is a particular need for practical compositions and methods for delivering PDGF or other growth factors to internal sites, such as bone. Such compositions should protect the growth factor(s) from proteolytic degradation and release the active polypeptide(s) over a period of time of days, weeks, or months, thus eliminating the need for daily administration. The present invention provides such compositions and also provides other, related advantages.

Summary of the Invention

The present invention provides sustained release compositions for the therapeutic delivery of polypeptide growth factors, such as PDGF, TGF-α, IGF I, bFGF, aFGF and EGF. The compositions are in the form of a biodegradable film which can be affixed to an implantable or prosthetic

device, such as a surgical pin, screw, plate or the like. One aspect of the present invention provides biodegradable films which comprise a polylactic acid-polyglycolic acid copolymer having a ratio of polylactic acid:polyglycolic acid between 70:30 and 30:70, one or more polypeptide growth factors, and a carrier selected from the group consisting of albumin, glutamic acid, and polyoxyethylene-sorbitan detergents. Within one embodiment, the biodegradable film comprises platelet derived growth factor. The film may further comprise insulin-like growth factor I or transforming growth factor beta.

Within a related aspect, the invention provides implantable and prosthetic devices having an outer surface, wherein a biodegradable film as described above is affixed to the outer surface. Implantable devices of this type include screws, pins, plates, rods, artificial joint components and bone filling materials. The devices may themselves be biodegradable.

The films and implantable devices of the present invention are useful within methods for enhancing repair of bone fractures in animals, wherein such a film or device is applied to a fractured bone of an animal at the fracture site. Within one embodiment, the film is affixed to an outer surface of an implantable or prosthetic device, and the device is applied to the fractured bone.

These and other aspects of the present invention will become evident upon reference to the following detailed description of the invention and the attached drawings.

Brief Description of the Drawings

Figure 1 illustrates the degradation over time of 100% PLA, 50:50 PLA/PGA and 85:15 PLA/PGA films *in vitro* as determined by mass loss.

Figure 2 illustrates the *in vitro* degradation of 50:50 PLA/PGA copolymer films as determined by gel permeation chromatography.

Figure 3 illustrates the *in vitro* degradation of 85:15 PLA/PGA copolymer films as determined by gel permeation chromatography.

Figure 4 illustrates the release of PDGF from PLA/PGA copolymer films with and without albumin.

Figure 5 illustrates the release of PDGF from PLA/PGA copolymer films.

Detailed Description of the Invention

The present invention provides compositions for the sustained release of polypeptide growth factors in the body of a patient. The compositions are in the form of biodegradable polyester films, such as polylactic acid, polyglycolic acid, polydioxanone or polylactic acid/polyglycolic acid copolymer films. Within a preferred embodiment, the films comprise a polylactic acid-polyglycolic acid copolymer, one or more polypeptide growth factors, and a carrier such as albumin, glutamic acid, or a polyoxyethylenesorbitan detergent.

Polylactic acid-polyglycolic acid (PLA/PGA) copolymers are prepared according to procedures known in the art. See, for example, Loomis et al., U.S. Patent No. 4,902,515; Gilding and Reed, Polymer 20: 1459-1464, 1979; and Boswell et al., U.S. Patent No. 3,773,919, which are incorporated herein by reference in their entirety. In general, polylactic acid, polyglycolic acid and copolymers thereof are commercially available. Typically, copolymer films are produced by combining the desired amount of PLA/PGA copolymer granules in a suitable solvent (e.g. chloroform or methylene chloride), pouring the resulting solution into a mold, and completely evaporating the solvent. In the alternative, PLA/PGA films may be produced by compression molding, extrusion, or other known methods.

As used herein the term "copolymer" includes any polymer containing two or more types of monomer unit. Copolymers may be classified in four types as shown in the

following chart, wherein "A" and "B" denote the component monomer units:

Random: -A-B-A-A-B-A-B-B-B-A-A-B-

Alternating: -A-B-A-B-A-B-A-B-A-B-A-B-

Block: -A-A-A-A-A-B-B-B-B-B-B-A-A-

Graft:

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-A-A-A-A-A-A-A-A-A-A-A-A-
      |           |
      B           B
      |           |
      B           B
      |           |
      B           B
      |           |
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Within the present invention, random copolymers are generally preferred as they are less crystalline and therefore degrade more quickly than other types of copolymers.

Because polymers of enantiomeric lactides are crystalline and therefore more resistant to degradation than their racemic counterparts, it is preferred to use mixed enantiomer (e.g. poly (D,L-lactic acid)) polymers within the present invention.

PLA/PGA films of the present invention are formulated to provide a ratio of PLA:PGA between 70:30 and 30:70, preferably between 65:35 and 35:65, more preferably between 55:45 and 45:55, and most preferably 50:50.

Degradation of the film and consequent release of growth factors therefrom can be modulated by adjusting such film parameters as molecular weight, copolymer structure, copolymer ratio and thickness. In general, the film will be formulated using a copolymer having a molecular weight between 10,000 and 200,000 Daltons. Film thicknesses of less than about 50 μm are preferred, particularly film thicknesses between 5 and 20 μm. In general, lower molecular weight, random copolymers will degrade more rapidly than higher molecular weight formulations or block copolymers. As illustrated in Figure 1, a 40-50 μm film of 50:50 PLA/PGA random

copolymer of ~100,000 molecular weight has been found to lose 95% of its mass after incubation for 76 days in 0.1 M sodium phosphate buffer, pH 7.4, at 37°C. PDGF was released from films of this type over at least 30 days when albumin was included as a carrier.

Polypeptide growth factors suitable for use within the present invention include PDGF, TGFα, TGFβ, IGF I, bFGF, aFGF, EGF and the like. Growth factors may be included in the compositions of the present invention singly or in combination. For example, combinations of PDGF and TGFα have been found to be useful in wound healing (Antoniades et al., U.S. Patent No. 4,874,746). Methods for producing polypeptide growth factors are known in the art. See, for example, U.S. Patents Nos. 4,783,412; 4,885,163; 4,889,919; 4,956,455 and 5,045,633, and European Patent Office Publication 200,341 A1, which are incorporated herein by reference.

A particularly preferred polypeptide growth factor is PDGF. In the description of the invention which follows, PDGF is disclosed as representative of the polypeptide growth factors. Those skilled in the art will recognize that other growth factors may be substituted for or used in combination with PDGF.

Within the context of the present invention, PDGF will be understood to include the AA, BB, and AB isoforms of PDGF, individually or in combination, as well as biologically active analogs thereof. In addition, the BB isoform of PDGF is understood to encompass the viral homolog (the v-sis gene product). PDGF may be obtained from either native or recombinant sources. Methods for producing recombinant PDGF and PDGF analogs are described within U.S. Patents Nos. 4,769,322; 4,801,542; and 4,766,073 and within EP 282,317, which are incorporated herein by reference in their entirety. PDGF may also be produced in bacteria (See Tackney et al., WO 90/04035). Methods for purifying PDGF from native sources are described by Raines and Ross (J. Biol. Chem. 257: 5154-

5160, 1982), Hart et al. (Biochemistry 29: 166-172, 1990), and in U.S. Patent No. 4,479,896.

As discussed in certain of the issued patents noted above, it has been found that by utilizing the secretory pathway of eucaryotic cells to express recombinant PDGF, biologically active material may be obtained directly. Expression and secretion of the appropriate gene product from eucaryotic cells enables proper processing and assembly, resulting in molecules with a native and biologically active conformation. Provided that appropriate transcriptional promoter and secretory signal sequences are utilized, generally any eucaryotic cell can express and secrete PDGF in a biologically active form for use within the present invention. In the alternative, PDGF polypeptide chains can be expressed in procaryotic cells, isolated, and assembled *in vitro* to produce biologically active molecules.

For expression of PDGF in yeast, a DNA sequence encoding a PDGF polypeptide (e.g. PDGF A chain or PDGF B chain) is ligated to an appropriate promoter and secretory signal sequence. Promoters which may be utilized in yeast include the yeast alpha-factor (MFα1) promoter and the yeast triose phosphate isomerase (TPI1) promoter (U.S. Patent No. 4,559,311). Promoters may also be obtained from other yeast genes, e.g., alcohol dehydrogenase I (ADH1) or alcohol dehydrogenase 2 (ADH2). Appropriate promoters for other eucaryotic species may also be used and will be apparent to those skilled in the art. Secretion of the PDGF gene products may be accomplished through use of the prepro secretory signal sequence of the yeast mating pheromone alpha-factor (Kurjan and Herskowitz, Cell 30: 933, 1982; Julius et al., Cell 36: 309, 1984; and Brake et al., Proc. Natl. Acad. Sci. USA 81: 4642, 1984), or the yeast BAR1 gene leader and third domain sequences (see U.S. Patent No. 5,037,743), although other secretion signals may be used. To ensure the efficient transcription termination and polyadenylation of

mRNA, a yeast terminator sequence, such as the triose phosphate isomerase terminator, may be added (Alber and Kawasaki, J. Molec. Appl. Genet. 1: 419, 1982). Methods of ligation of DNA fragments have been amply described (Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory Press, 1989) and are well within the level of ordinary skill in the art. After preparation of the expression unit constructs, they are inserted into an appropriate expression vector.

It is preferable to use an expression vector which is stably maintained within the host cell in order to produce more biological activity per unit of culture. Suitable yeast expression vectors in this regard are the plasmids pCPOT (ATCC 39685) and pMPOT2 (ATCC 67788), which include the Schizosaccharomyces pombe gene encoding the glycolytic enzyme triose phosphate isomerase (POT1 gene). Inclusion of the POT1 gene ensures the stable maintenance of the plasmid in a host cell having a TPI gene deletion due to its ability to complement the gene deletion in the host cell, as disclosed in U.S. Patent No. 4,931,373, which is incorporated herein by reference.

After preparation of a DNA construct incorporating the POT1 selectable marker and an expression unit comprising, for example, the TPI1 promoter, the BAR1 leader and third domain sequences, an appropriate DNA sequence encoding PDGF, and the TPI1 terminator, the construct is transformed into a yeast host with a TPI1 gene deletion. Procedures for transforming yeast are well known and have been described in the literature.

The transformed yeast cells may be selected by growth on a conventional complex medium containing glucose when the POT1 gene is utilized as a selectable marker. A conventional medium, such as YEPD (20 grams glucose, 20 grams Bacto-peptone, 10 grams yeast extract per liter), may be used. Once selected, transformants containing the appropriate expression constructs are grown to stationary phase on conventional complex media, the cells removed by

centrifugation or filtration, and the medium concentrated. Since PDGF is a highly cationic and hydrophobic protein (Raines and Ross, ibid.; Antoniades, Proc. Natl. Acad. Sci. USA 78: 7314, 1981; Deuel et al. J. Biol. Chem. 256: 8896, 1981), recombinant PDGF similarly possesses characteristics which allow the use of ion exchange chromatography in its purification. For example, recombinant PDGF-BB in yeast fermentation broth is separated from the cells and fractionated by cation exchange chromatography. PDGF-BB desorbed from the column is acidified and further fractionated by reverse phase chromatography under batch conditions. The PDGF-containing effluent is acidified and passed through a strong cation exchange column and eluted with a NaCl step gradient. The effluent is collected, and PDGF-BB is precipitated using $(NH_4)_2SO_4$. The resulting material is desalted by gel filtration and separated according to charge. The effluent is acidified and applied to a strong cation exchange column and eluted with a linear gradient of NH_4HCO_3 at pH 8-10. The effluent is collected, and the PDGF-BB is precipitated by the addition of $(NH_4)_2SO_4$. The resulting precipitate is dissolved in acetic acid and fractionated by gel filtration. The effluent is desalted and lyophilized.

Expression of biologically active proteins in eucaryotic cells other than yeast cells can be achieved by a person skilled in the art through use of appropriate expression/regulatory signals. Transcriptional promoters capable of directing the expression of PDGF sequences are chosen for their ability to give efficient and/or regulated expression in the particular eucaryotic cell type. Signal sequences capable of directing the gene product into the cell's secretory pathway are chosen for their function in the host cell. The selection of other useful regulatory signals, such as transcription termination signals, polyadenylation signals and

transcriptional enhancer sequences, will be apparent to an individual skilled in the art.

Recombinant PDGF has been shown to possess substantially the same biological activity as native PDGF. The basic biological activity of PDGF, particularly the induction of chemotaxis and mitogenesis in responsive cell types (including fibroblasts and smooth muscle cells), underlies many of the physiological roles of this protein, including its role in tissue repair.

Growth factors that are utilized within the present invention are preferably substantially pure, that is, generally free of impurities or contaminants which would interfere with their therapeutic use. Particularly preferred are those preparations which are free of toxic, antigenic, inflammatory, pyrogenic or other deleterious substances, and are greater than 90%, preferably greater than 99%, pure.

Release of biologically active growth factors from the films of the present invention is enhanced by including a carrier such as albumin, a polyoxyethylenesorbitan detergent or glutamic acid. In principle, any substance that enhances polymer degradation, creates pores in the film or reduces adsorption of the growth factor(s) to the film can be used as a carrier. Albumin is a particularly preferred carrier. Polyoxyethylenesorbitan detergents that are useful as carriers include polyoxyethylenesorbitan monooleate, polyoxyethylenesorbitan monolaureate, polyoxyethylenesorbitan monopalmitate, polyoxyethylenesorbitan monostearate and polyoxyethylenesorbitan trioleate.

In addition to the copolymers, growth factors and carriers noted above, the biodegradable films may include other active or inert components. Of particular interest are those agents that promote tissue growth or infiltration. Agents that promote bone growth, such as bone morphogenic proteins (U.S. Patent No. 4,761,471; PCT

Publication WO 90/11366), osteogenin (Sampath et al., Proc. Natl. Acad. Sci. USA 84: 7109-7113, 1987) and NaF (Tencer et al., J. Biomed. Mat. Res. 23: 571-589, 1989) are particularly preferred.

To load the films, a therapeutically effective amount of one or more growth factors and a carrier are applied to the film as powders or liquid solutions. For example, lyophilized PDGF and albumin may be uniformly dispersed over one surface of the film, and the film folded over. In the alternative, the proteins may be applied as aqueous solutions (e.g., in phosphate buffered saline or 0.1 M acetic acid), which are allowed to dry.

A "therapeutically effective amount" of a growth factor is that amount sufficient to provide a significant increase in healing over the course of treatment. Determination of such amounts is within the level of ordinary skill in the art and will generally be based on *in vitro* and *in vivo* models of wound healing. When PDGF is included in the film, it will typically be provided in an amount between 0.0375 and 1.25 μg per mg of copolymer, although greater or lesser amounts may be used depending on the degradation and release characteristics of the film. It is preferred to provide at least about 0.125 μg of PDGF per mg of copolymer.

When albumin is used as a carrier, it will generally be included at between 0.1 and 1.0 mg per mg of copolymer, preferably 0.25-0.5 mg per mg of copolymer, although lesser or greater amounts may be used to retard or enhance growth factor release. In any event, it is preferred to maintain the ratio of PDGF to albumin between 0.125 and 2.5 μg/mg. Glutamic acid may also be used as a carrier at between about 0.05 and 2.0 mg per mg of copolymer, although greater or lesser amounts may be used as necessary to obtain the desired rate of growth factor release. Polyoxyethylenesorbitan detergents will generally be used at between 0.05 and 0.25 μl per mm^2 of the film surface.

After loading with growth factor and carrier, the film is sterilized. Sterilization by exposure to a sterilizing gas, such as cold ethylene oxide or chlorine dioxide is preferred, although other sterilization methods that do not cause denaturation of the growth factor(s) or breakdown of the polymer film may be employed. Following preparation, the films are stored refrigerated (e.g. 4°C) until use.

The biodegradable films of the present invention are particularly useful as coatings for prosthetic devices and surgical implants. The films may, for example, be wrapped around the outer surfaces of surgical screws, rods, pins, plates and the like. Implantable devices of this type are routinely used in orthopedic surgery. Of particular interest are screws and rods made of biodegradable materials such as PLA and/or PGA. The films can also be used to coat bone filling materials, such as hydroxyapatite blocks, demineralized bone matrix plugs, collagen matrices and the like, or applied to the surfaces of prosthetic devices (e.g. components of artifical joints) to promote tissue ingrowth.

The films and devices of the present invention are useful within methods for promoting tissue growth and repair. Of particular interest is the repair of bone fractures in animals. In this regard, a film or device as described herein is applied to the bone at the fracture site. Application is generally by implantation into the bone or attachment to the surface using standard surgical procedures. Biodegradable films according to the present invention are also useful for stimulating vascularization and promoting the growth of soft tissue. For example, a film may be fashioned into a sleeve around a damaged ligament.

These and other uses of the biodegradable films of the present invention are within the level of ordinary skill in the art. In general, the films will find utility in any wound healing application where it is advantageous to provide a continuous supply of a growth factor over an extended period of time or in situations where it is difficult to provide multiple applications of growth factors.

The films of the present invention are particularly useful in individuals who have substantially impaired wound healing capacity, and thereby lack the ability to provide to the wound site endogenous growth factors which are necessary for the process of wound healing. In these individuals, the addition of exogenous growth factors enables wound healing to proceed in a normal manner. Normal wound-healing may be retarded by a number of factors, including advanced age, diabetes, cancer, and treatment with anti-inflammatory drugs or anticoagulants, and thus the therapeutic activity of exogenous growth factors may be used to offset the delayed wound-healing effects of such diseases and treatments.

The following examples are offered by way of illustration, not by way of limitation. It will be appreciated by those skilled in the art that the films disclosed within the examples may, for example, be used with other growth factors or other isoforms and analogs of PDGF.

Example 1

Recombinant PDGF-BB was produced in yeast host strain E18#9 (an **a**/α diploid homozygous for Δtpi) as generally disclosed in U.S. Patent No. 4,845,075 using the BAR1 leader and third domain sequences to direct secretion as disclosed in U.S. Patent No. 5,037,743. Cells were fermented in a yeast extract - dextrose medium containing trace metals. Secreted PDGF was purified by a combination

of cation exchange chromatography, gel filtration and ammonium sulfate precipitation.

Polylactic acid and polylactic acid-polyglycolic acid films were solvent cast by dissolving approximately 340 mg of polymer granules (Medisorb Technologies International L.P, Wilmington, DE or Polysciences, Warrington, PA) in 10 ml chloroform at room temperature and allowing the solvent to evaporate completely in a slow air flow hood at room temperature. Films were produced using 100% PLA and PLA/PGA mixtures of 85:15 and 50:50. The films were on the average between 40 and 50 μm thick. Each was cut into a ca. 80 mm x 40 mm sheet, resulting in a remaining polymer mass of about 270-290 mg. The films were then rolled around 0.9 mm diameter Kirschner wires (K-wires), resulting in an implant diameter of 2.8-3.0 mm. *In vitro* degradation studies were carried out in 0.1 M Na phosphate buffer, pH 7.4, at 37°C. Triplicate specimens were removed from the buffer and vacuum dried at 11, 25, 53, 76, 150 and 250 days. The buffer solution was changed at each timepoint. Degradation over time was analyzed by mass loss and molecular weight distribution changes. Molecular weight was characterized by gel permeation chromatography (GPC, Waters HPLC 590) using dimethylacetonitrile (DMAC) as the solvent.

The unloaded *in vitro* degradation study showed mass loss from the 50:50 and 85:15 PLA/PGA copolymer rods in the range of 80-95% by the 76-day point, but virtually no mass loss for the 100% PLA implants. These results can be seen in Figure 1. The GPC results shown in Figures 2 and 3, and based on a polystyrene standard, confirm the degradation of the copolymer specimens, even over a short 25-day period. The molecular weight decrease, indicated by the shift of the peaks to the left at later timepoints, is by an order of magnitude in both cases. Due to the lack of degradation of the 100% PLA implant, no further characterization was carried out on these samples.

Example 2

An *in vitro* evaluation of the ability of various implant compositions to deliver active PDGF was also performed. Six specimens were fabricated as shown in Table 1.

Table 1

Specimen	PLA/ PGA	Polymer	Film size.	Film Thick.	Rod Diam.	PDGF (μg)	Albumin (mg)
E	50:50	246.8mg	83x42mm	30μm	3.4mm	102.4	37
F	85:15	250.2mg	81x41mm	50μm	3.7mm	100.4	40
G	50:50	259.6mg	86x41mm	40μm	3.0mm	114.3	0
H	85:15	250.0mg	87x41mm	45μm	2.9mm	113.9	0
I	50:50	300.5mg	83x43mm	20,17μm	3.5mm	95.4	41
K	50:50	364.3mg	90x46mm	30μm	3.0mm	97.9	0

All rods except specimen K were loaded with lyophilized PDGF and albumin by spreading the amount of powder indicated above onto half of the film, and folding the other side over it, thus creating a sandwich. The edges of the film were pressed together to eliminate excessive loss of PDGF and albumin, and the film was then rolled around the K-wire into the implant rod configuration.

Specimen K was solvent cast with methylene chloride rather than chloroform. Both 50:50 PLA/PGA and PDGF were dissolved into the methylene chloride, and the resulting film was made into a rod as above without any additional carrier or growth factor loading.

The implants were subjected to a temperature of 37°C in sterilized pH 7.4 phosphate-buffered saline. 1 ml samples were taken at 27 timepoints over a span of 40 days. 100 μl of an acetic acid solution was added to each sample to stabilize the PDGF until assays could be performed. The buffer was changed after every timepoint,

so all PDGF observed in solution was released only after the previous timepoint.

The amount of PDGF released by each implant was determined by an enzyme-linked immunosorbent assay (ELISA) as generally described in U.S. Patent No. 5,094,941, using an anti-PDGF monoclonal first antibody and a rabbit anti-PDGF polyclonal second antibody. PDGF was quantitated using goat anti-rabbit IgG coupled to horseradish peroxidase. A standard of known concentration was run on each plate and curve-fit to a straight line on a semi-log graph. The concentration of PDGF was calculated by plugging the observed reading into the standard curve equation for that plate. The cumulative amount released was back calculated from the individual sample concentrations and added together.

The release characteristics of the various implants used in the PDGF-loaded *in vitro* study are shown in Figures 4-5. Figure 4 shows the release curves of four of the rods--two each of 50:50 PLA/PGA and 85:15 PLA/PGA, one of each set with rabbit albumin (RA) and one without it. Faster initial release and overall greater PDGF release was observed for the specimens with RA. The less crystalline nature of 50:50 PLA/PGA also enhanced the degradation, and thus the maximum PDGF release was observed from rod E, composed of the 50:50 copolymer loaded with both PDGF and RA.

Even greater release was observed from the 50:50 PLA/PGA implant loaded with PDGF and RA but constructed with thinner film (rod I). This is shown in Figure 5. The comparison between four specimens, all fabricated with 50:50 PLA/PGA, shows that thinner films and the presence of albumin both enhance the initial and overall release of PDGF. Rod K, made by dissolving both the polymer and PDGF in methylene chloride, did not give any significant release of active PDGF.

Example 3

PLA/PGA (50:50) films were solvent cast as described in Example 1 to give a thickness of ~10 μm. PDGF and rabbit serum albumin were dispersed on the films as a 0.1 M acetic acid solution, and the liquid was allowed to evaporate. The films were then rolled around K-wires to provide implants of 1.5 or 3.0 mm diameter as shown in Table 2. Five implants of each series were prepared.

Table 2

Implant Diameter	PDGF (μg)	Albumin (mg)
1.5 mm	100	40
3.0 mm	10	40
	100	40

The implants were sterilized using cold ethylene oxide gas. Individual implants were immersed in vials of phosphate buffered saline, pH 7.4, and held at 37°C in a waterbath. The contents of the vials are assayed at intervals to determine rates of PDGF release.

Example 4

PLA/PGA (50:50) films are prepared and loaded as described in Example 3. Films are rolled around 0.6 mm diameter steel K-wires (Zimmer, Warsaw, IN). The resulting pins are cut to a length of 2 cm. The pins are implanted into one femur of each of eight rats. Control rats receive sham implants lacking PDGF. Four weeks after implantation, bones are harvested and evaluated for mineralized bone density/area, bending strength and histology.

Example 5

PLA/PGA (50:50) films, with and without PDGF, are prepared as described in Example 3. Two sets of five dynamic compression plates (DCP) are prepared by attaching PLA/PGA film by multiple layered coatings to appropriately sized stainless steel DCP. The PDGF-loaded films are constructed and applied to the plates so that PDGF is on only one side of the plate.

Rabbits are anesthetized, and a midshaft osteotomy is created in the right femur of each animal. The film-coated dynamic compression plates are fixed to the femurs with screws located proximal and distal to the osteotomy with the PDGF side adjacent to the bone. The left leg of each animal is left intact. Six animals are provided with PDGF-loaded plates, and six are provided with sham implants constructed identically but with the film loaded with only rabbit albumin.

Fluorochlorine labels are given and radiographs taken to determine growth rates.

At six and twelve weeks after surgery, bones are harvested, tested biomechanically to failure, and subjected to histomorphometric analysis.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for the purpose of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

We claim:

1. A biodegradable film comprising a polylactic acid-polyglycolic acid copolymer having a ratio of polylactic acid:polyglycolic acid between 70:30 and 30:70, a therapeutically effective amount of a polypeptide growth factor, and a carrier selected from the group consisting of albumin, glutamic acid and polyoxyethylenesorbitan detergents.

2. A biodegradable film according to claim 1 wherein the ratio of polylactic acid:polyglycolic acid is between 65:35 and 35:65.

3. A biodegradable film according to claim 1 wherein the ratio of polylactic acid:polyglycolic acid is between 55:45 and 45:55.

4. A biodegradable film according to claim 1 wherein the ratio of polylactic acid:polyglycolic acid is 50:50.

5. A biodegradable film according to claim 1 wherein said carrier is albumin.

6. A biodegradable film according to claim 5 wherein said albumin is present at between 0.1 and 1.0 mg per mg of copolymer.

7. A biodegradable film according to claim 5 wherein said albumin is present at between 0.25 and 0.5 mg per mg of copolymer.

8. A biodegradable film according to claim 1 wherein said growth factor is platelet derived growth factor, transforming growth factor alpha, insulin-like growth factor I, basic fibroblast growth factor, acidic fibroblast growth factor or epidermal growth factor.

9. A biodegradable film according to claim 1 wherein said growth factor is platelet derived growth factor.

10. A biodegradable film according to claim 9 wherein said platelet derived growth factor comprises the BB isoform of platelet derived growth factor.

11. A biodegradable film according to claim 9 wherein said platelet derived growth factor is present at between 0.0375 and 1.25 μg per mg of copolymer.

12. A biodegradable film according to claim 9 further comprising insulin-like growth factor I or transforming growth factor beta.

13. A biodegradable film according to claim 9 wherein said carrier is albumin and wherein the ratio of PDGF:albumin is between 0.125 and 2.5 μg/mg.

14. A biodegradable film according to claim 1 having a thickness of about 5 to 50 μm.

15. A biodegradable film according to claim 1 having a thickness of about 5 to 20 μm.

16. A biodegradable film according to claim 1 wherein said copolymer has a molecular weight of from 10 kDa to 200 kDa.

17. A biodegradable film according to claim 1 further comprising a bone morphogenic protein, osteogenin, or NaF.

18. A biodegradable film according to claim 1 wherein said copolymer is a random copolymer.

19. An implantable or prosthetic device having an outer surface, wherein a biodegradable film comprising a polylactic acid-polyglycolic acid copolymer having a ratio of polylactic acid:polyglycolic acid between 70:30 and 30:70, a therapeutically effective amount of a polypeptide growth factor, and a carrier selected from the group consisting of albumin, glutamic acid and polyoxyethylenesorbitan detergents is affixed to said outer surface.

20. An implantable device according to claim 19 wherein said device is a screw, pin, plate, rod or artificial joint component.

21. An implantable device according to claim 19 wherein said device is a bone filling material.

22. An implantable device according to claim 21, wherein said device is a hydroxyapatite block.

23. An implantable device according to claim 19 wherein said device is biodegradable.

24. An implantable device according to claim 19 wherein said growth factor is platelet derived growth factor.

25. An implantable device according to claim 19 wherein said carrier is albumin.

26. A biodegradable film comprising:

a polylactic acid-polyglycolic acid copolymer having a ratio of polylactic acid:polyglycolic acid between 55:45 and 45:55;

a therapeutically effective amount of platelet derived growth factor; and

albumin.

27. A method for enhancing repair of a bone fracture in an animal comprising applying to a fractured bone of an animal at the fracture site a biodegradable film comprising a polylactic acid-polyglycolic acid copolymer having a ratio of polylactic acid:polyglycolic acid between 70:30 and 30:70, a therapeutically effective amount of a polypeptide growth factor, and a carrier selected from the group consisting of albumin, glutamic acid and polyoxyethylenesorbitan detergents.

28. A method according to claim 27 wherein said growth factor is platelet derived growth factor.

29. A method according to claim 27 wherein said film is affixed to an outer surface of an implantable or prosthetic device, and said device is applied to said fractured bone.

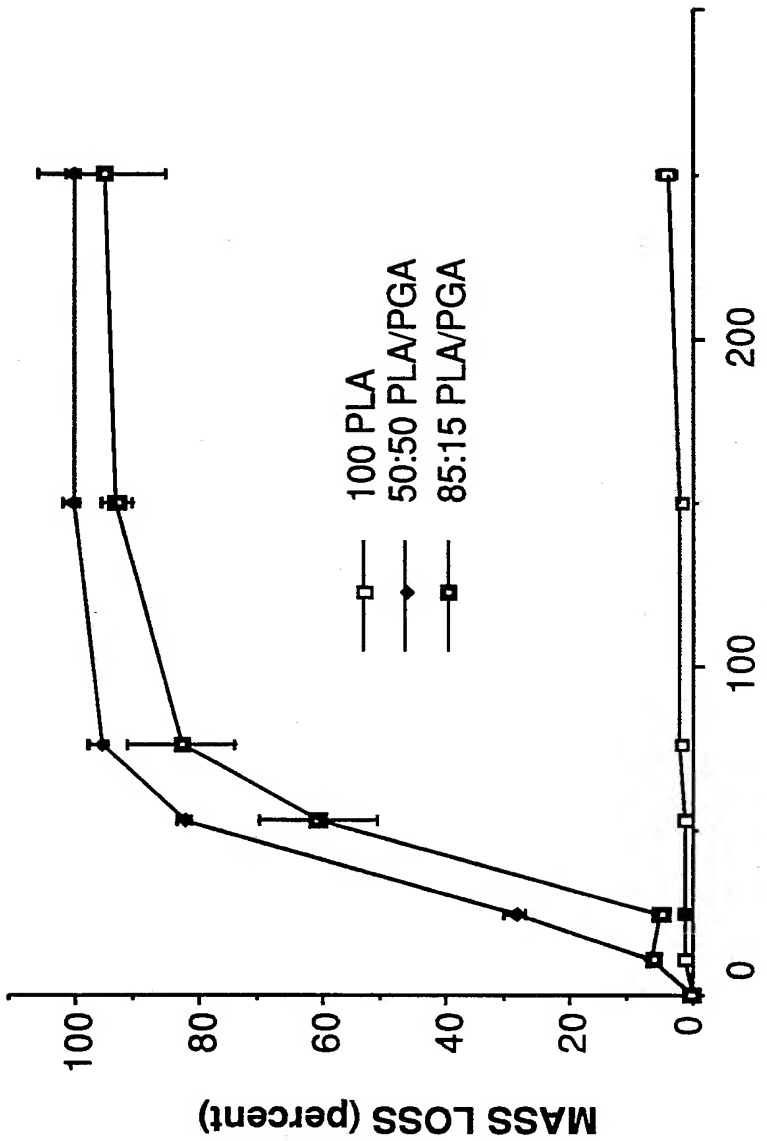
MASS LOSS (percent)
0
20
40
60
80
100
0
100
200
TIME (days)
100 PLA
50:50 PLA/PGA
85:15 PLA/PGA

FIGURE 1

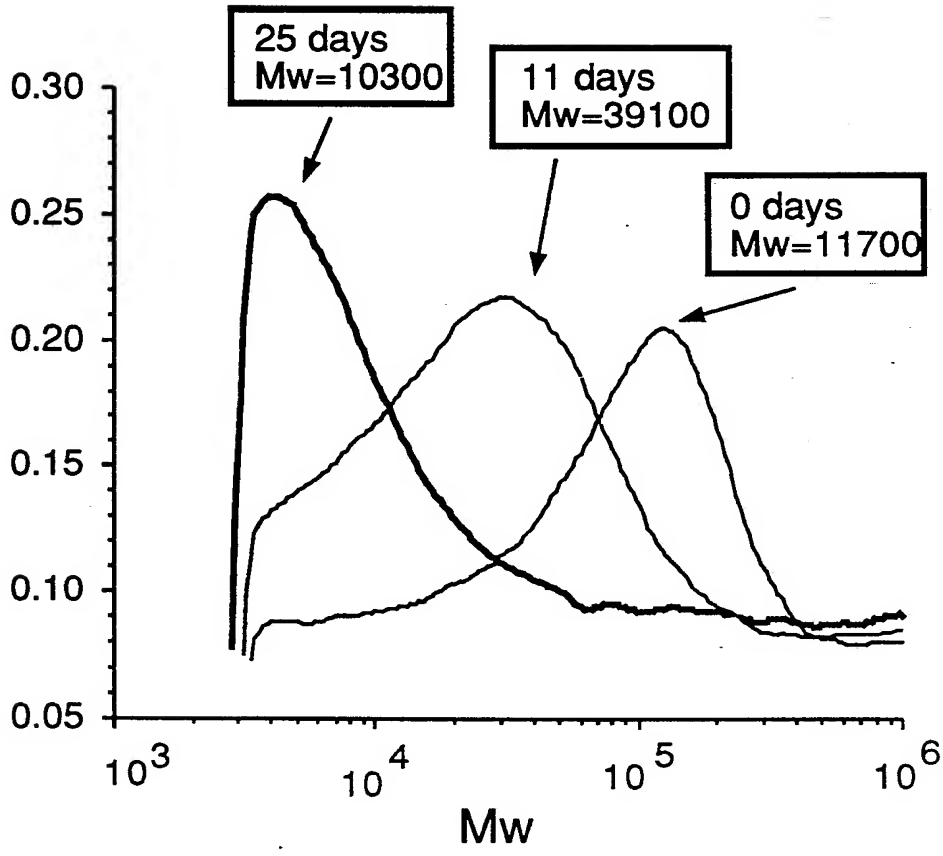
25 days
Mw=10300
11 days
Mw=39100
0 days
Mw=11700
0.30
0.25
0.20
0.15
0.10
0.05
10^3
10^4
10^5
10^6
Mw

FIGURE 2

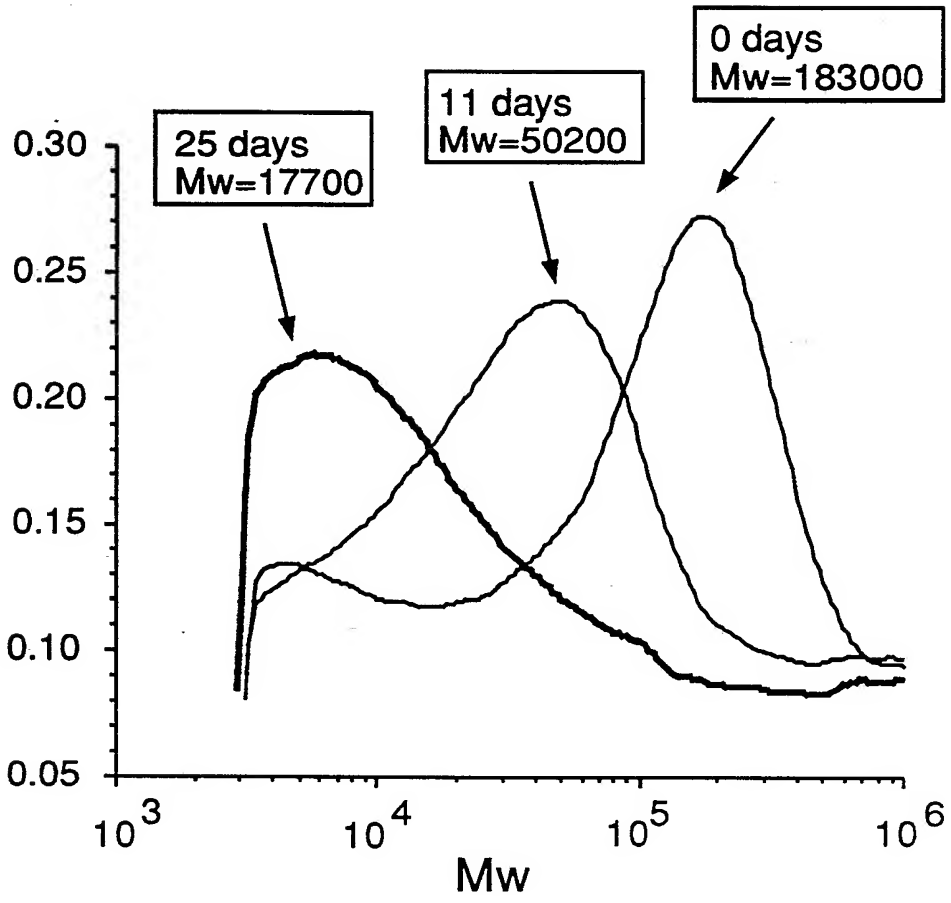
0 days
Mw=183000
11 days
Mw=50200
25 days
Mw=17700
0.30
0.25
0.20
0.15
0.10
0.05
10^3
10^4
10^5
10^6
Mw

FIGURE 3

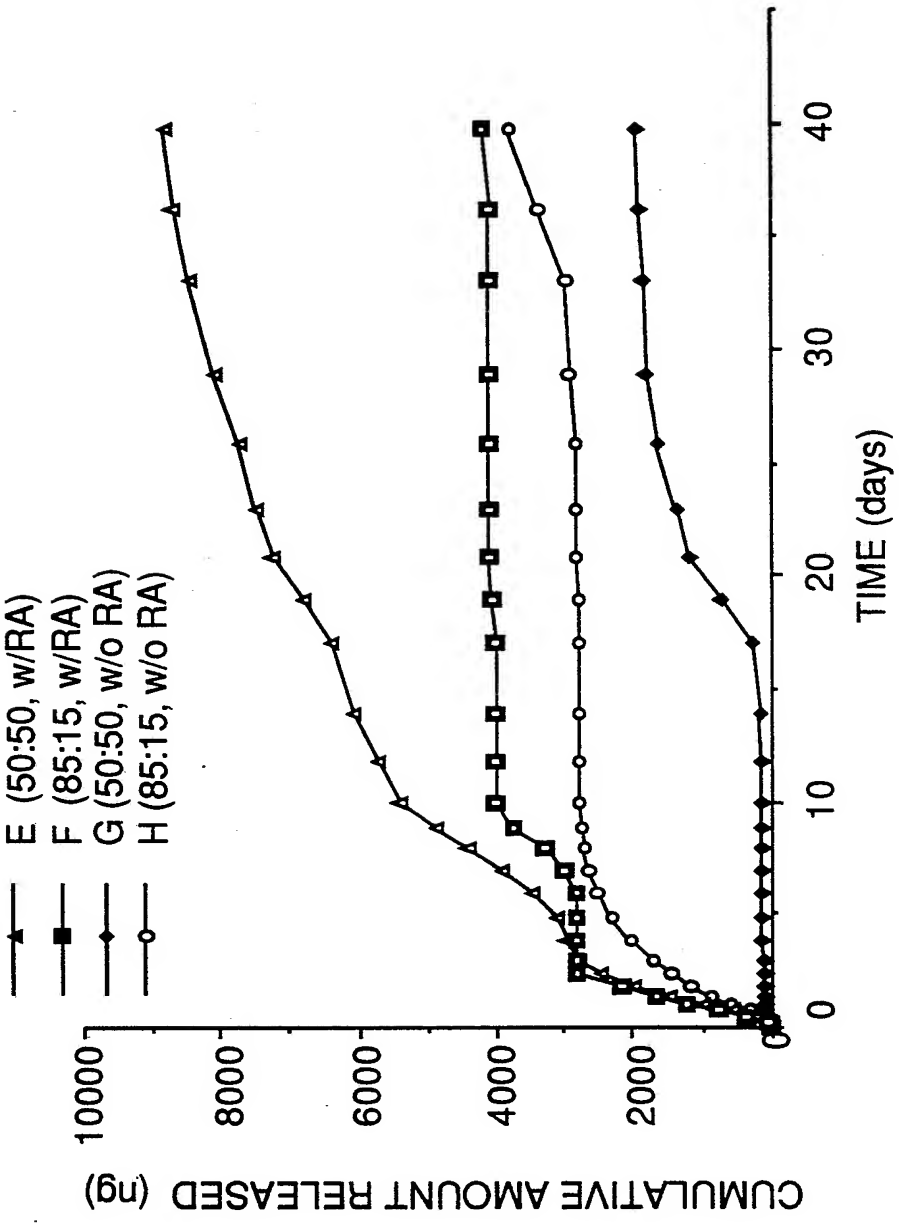
E (50:50, w/RA)
F (85:15, w/RA)
G (50:50, w/o RA)
H (85:15, w/o RA)
CUMULATIVE AMOUNT RELEASED (ng)
10000
8000
6000
4000
2000
0
0
10
20
30
40
TIME (days)

FIGURE 4

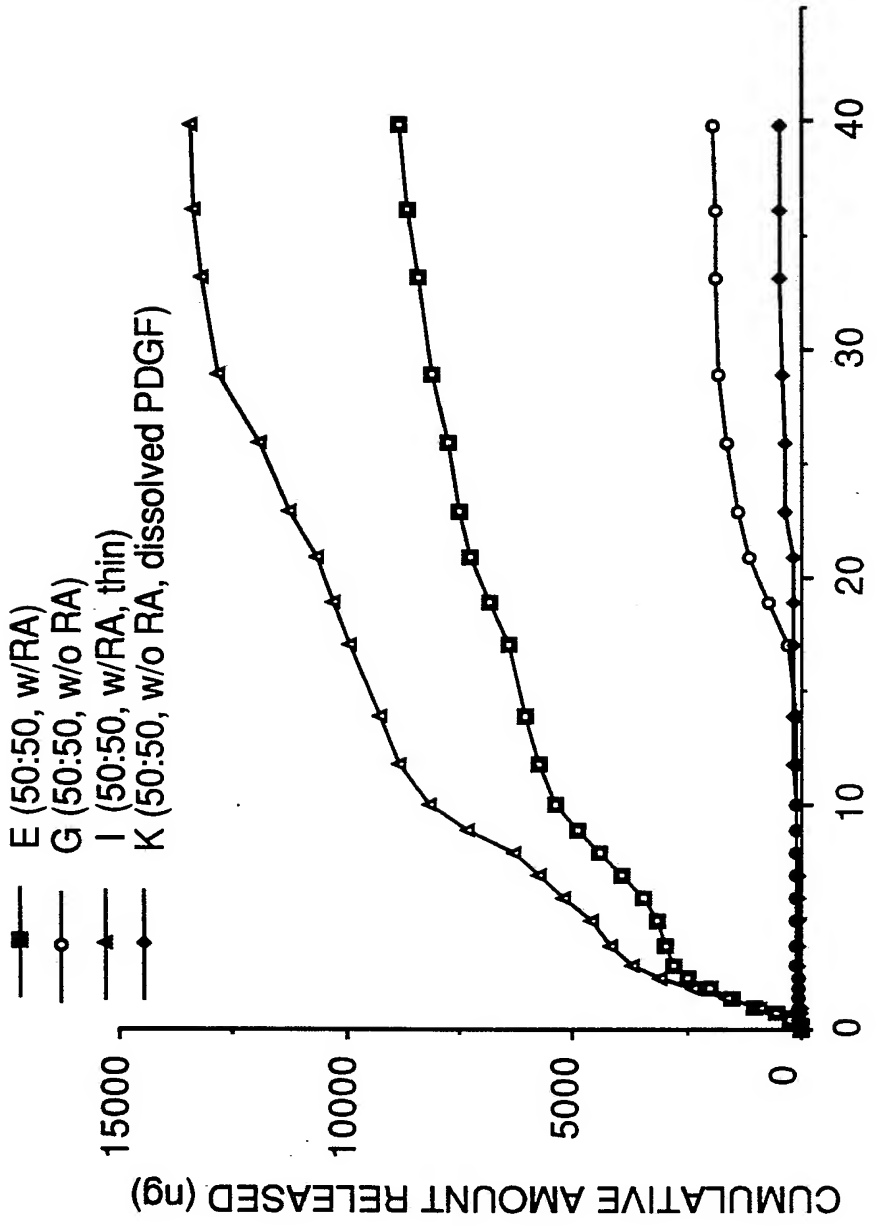
E (50:50, w/RA)
G (50:50, w/o RA)
I (50:50, w/RA, thin)
K (50:50, w/o RA, dissolved PDGF)
CUMULATIVE AMOUNT RELEASED (ng)
15000
10000
5000
0
0
10
20
30
40
TIME (days)

FIGURE 5

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 93/03648

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A61L31/00

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	A61L ; C08L

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
Y	US,A,4 902 515 (G. LOOMIS) 20 February 1990 cited in the application see column 9, line 22 - line 23; claims; example 1	1-29
Y	GB,A,2 215 209 (OSMED INC) 20 September 1989 see page 4, line 1 see page 13, line 6 - line 11 see page 14, line 15 - page 15, line 13 see claims	1-18, 26-29
Y	EP,A,0 205 997 (DR. MÜLLER-LIERHEIM KG BIOLOGISCHE LABORATORIEN) 30 December 1986 see claims; figure	19-25

-/--

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
16 JULY 1993	26. 07. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	G.COUSINS-VAN STEEN

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	WO,A,9 005 522 (P. PRISELL) 31 May 1990 ---	
A	WO,A,9 013 302 (BRIGHAM AND WOMEN'S HOSPITAL) 15 November 1990 -----	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/03648

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 27-29 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9303648
SA 73326

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 16/07/93

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A-4902515	20-02-90	US-A-	4800219	24-01-89
		EP-A-	0438426	31-07-91
		JP-T-	4501109	27-02-92
		WO-A-	9003783	19-04-90
		US-A-	4981696	01-01-91
GB-A-2215209	20-09-89	JP-A-	1232967	18-09-89
		US-A-	5133755	28-07-92
EP-A-0205997	30-12-86	DE-A-	3521684	18-12-86
		DE-A-	3683321	20-02-92
		DE-A-	3687861	08-04-93
		EP-A,B	0205790	30-12-86
		JP-A-	62051984	06-03-87
		JP-A-	62049856	04-03-87
		US-A-	4828563	09-05-89
		US-A-	4789634	06-12-88
WO-A-9005522	31-05-90	AU-B-	632074	17-12-92
		AU-A-	4525389	12-06-90
		EP-A-	0444081	04-09-91
		SE-A-	8804164	17-11-88
WO-A-9013302	15-11-90	AU-A-	5654990	29-11-90